Heritability of Coral Calcification Rates and Potential for Adaptation to Ocean Acidification

A Senior Honors Project Submitted to the Faculty of the
Global Environmental Science
Undergraduate Division, University of Hawaiʻi at Mānoa

In Partial Fulfillment of the Requirements For
Bachelor of Science in Global Environmental Science with Honors

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December 2016

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Acknowledgements

This project was made possible by funding from the Undergraduate Research Opportunity Program and the National Science Foundation Grant NSF-OA#1416889. I would like to thank my advisor Dr. Robert Toonen for providing this rewarding opportunity and supporting my research endeavors. I would like to acknowledge and thank Dr. Christopher Jury for his guidance and support over the course of the project. It has been a pleasure to learn and gain laboratory research experience from such a passionate and experienced scientist. I would like to thank the Hawaiʻi Institute of Marine Biology for housing my research project, and the graduate students who provided boat transportation on and off Coconut Island. A special thanks to Dr. Michael Guidry for the moral support and reality checks- your guidance and reassurance made this possible. Finally, I would like to acknowledge my Global Environmental Science family for being so welcoming to me as a transfer student, and for the wonderful experiences throughout my time as an undergraduate.

Abstract

The increasing concentration of atmospheric carbon dioxide due to human activities is contributing to ocean acidification, which leads to a reduction in coral growth rates, but the mechanism by which this reduction occurs is unknown. Heritable variation underlies the potential for evolution over time, but the degree of variation in pH tolerances among corals, as well as the heritability of any such variation, was previously unknown. In this project, I calculated the heritability of calcification among eight of the dominant Hawaiian coral species under both ambient and low pH conditions predicted for the end of the century. Coral colonies were sampled across natural gradients in seawater chemistry from a total of six different locations around O‘ahu, Hawai‘i. Coral calcification response was assessed per colony by comparing growth in grams per mg per day between replicate samples in low and ambient pH water. Heritability was assessed using an R package to calculate the amount of variation in calcification rates that is due to genetics and may be passed on to offspring. The results show that calcification rates are highly heritable across all eight species, and all eight may experience selective pressure for calcification rate under acidification. *M. patula*, *P. meandrina*, and *P. evermanni* in particular show statistically significant variation in pH tolerance among colonies, making these especially good candidates for future studies on adaptation to ocean acidification. Further studies combining additional variables such as sea surface temperature and nutrient availability may lead to the creation of a holistic predictive model of Hawaiian reef composition in the future.

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1.0 Introduction

1.1 Ocean Acidification

Coral reefs house approximately one-quarter of marine biodiversity, protect coastlines from erosion, and sustain tourist economies, but these habitats are at risk as anthropogenic carbon dioxide emissions lower the pH of the ocean. Anthropogenic carbon dioxide levels have not exceeded 300ppm in the last 800,000 years according to ice core data provided by the Scripps Institute of Oceanography (SIO, 2016)(Figure 1). Today, levels are above 400ppm and rising at an average over 2ppm every year (ESRL, 2016). The oceans provide an important reservoir for the excess carbon in the atmosphere, taking up nearly 40% of anthropogenic carbon dioxide (CO_2) emissions since the industrial revolution (IPCC, 2014). However, this rapid rise in CO_2 absorption has significantly impacted surface ocean chemistry through a process called ocean acidification (OA).

Corals are calcifying marine animals that build their skeletons out of the dissolved carbonate mineral aragonite. Studies have shown a direct correlation between a decrease in pH, a decrease in aragonite saturation state, and a decrease in coral calcification (Jokiel et al. 2016). However, in some cases corals or other calcifying organisms seem to be insensitive, or even experience a small increase in calcification under moderate OA (Castillo et al. 2014). It cannot be said that OA is necessarily harmful to corals, but as the ocean is trending toward further acidification, it is important to study the magnitudes of OA effects on calcification rates for various coral species.

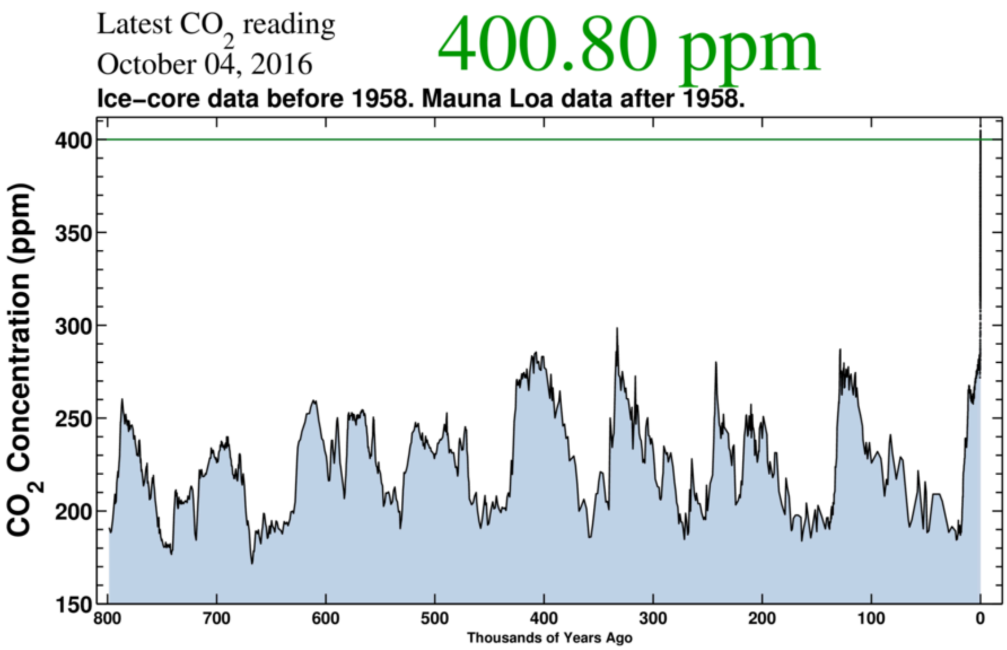


Figure 1. Global carbon dioxide concentration from the past 800,000 years. Source: Scripps Institute of Oceanography, 2016

1.2 Corals Physiological Response to Ocean Acidification

Most corals live in waters with an average pH level of ~8.1, which has already decreased by 0.1 units since the industrial revolution, and is projected to drop up to 0.4 units by 2100 (Caldeira and Wickett, 2003)(Figure 2). When the pH of the seawater decreases, corals face three possible responses: acclimatization, adaptation, or reduced performance. Acclimatization can occur in an individual coral over its lifetime. For example, if a coral is moved from a location of normal pH to a region of slightly lower pH, its physiology may change to acclimate to the environment. Adaptation is a process that occurs on the population level over one or more generations. In order for adaptation to occur, there must be heritable differences among the offspring and the favorable genes must be passed on. This process induces a population to change over time in response to environmental pressures. If corals are unable to acclimatize or adapt to new conditions they may survive, but experience chronically reduced growth rates.

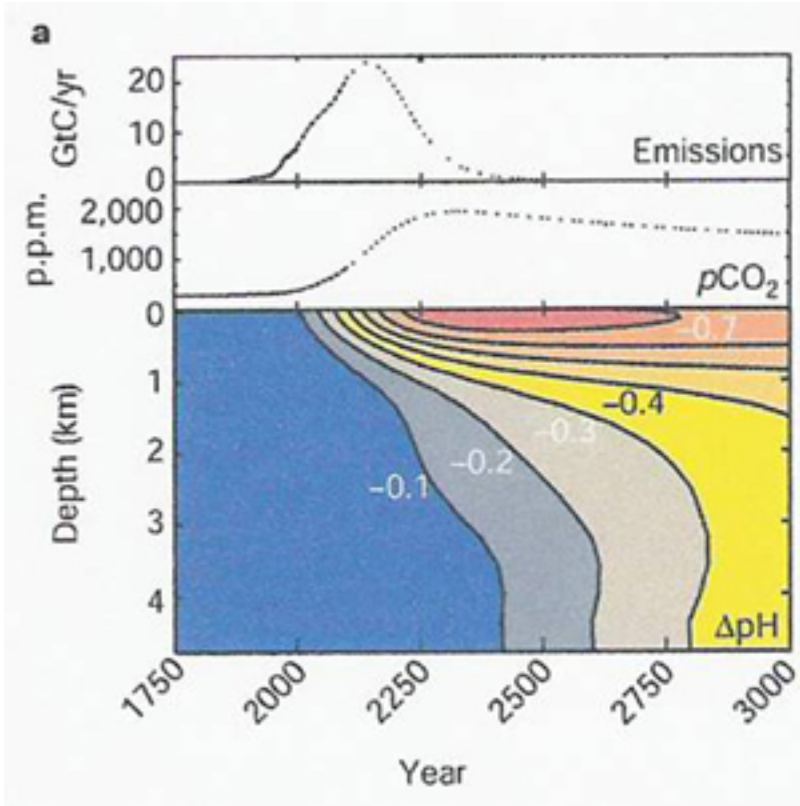


Figure 2. Projection of future surface ocean pH decrease. Source: Caldeira and Wickett**,** 2003

1.3 Previous Studies of Coral pH Tolerance

A coral is considered to be tolerant of low pH if it is able to grow at a rate comparable to conspecifics in ambient, or "normal", pH water. Recent work has shown that colonies of three coral species (*Montipora capitata*, *Pocillopora damicornis*, and *Porites compressa*) from Kāne'ohe Bay, O'ahu, live and grow under conditions of naturally higher acidity, or lower pH, than do colonies of the same species located elsewhere around the island (Bahr et al., 2016; Jury & Toonen, in review). *P. compressa* from the Bay show significantly higher calcification tolerances to low pH than do conspecifics growing in normal seawater chemistry whereas *P. damicornis* and *M. capitata* show relatively high pH tolerance across locations (Jury & Toonen, in review). This recent work suggests that some Hawaiian corals may be able to mount an adaptive response to ocean acidification.

1.4 Broad Sense Heritability

There is a distinction between narrow sense heritability and broad sense heritability. *Broad sense heritability* includes genetic, maternal, epigenetic, and other heritable sources of variation, whereas *narrow sense heritability* includes only the additive genetic component of heritable variation. Narrow sense heritability is typically assessed by contrasting individuals with know genetic relatedness (e.g., among siblings, half siblings, cousins, etc.) in controlled crossbreeding experiments so that the additive genetic component can be distinguished from other heritable effects. In this thesis, only broad sense heritability of pH tolerances was considered for the eight coral species. This allows for an estimation of the proportion of responses that may be attributed to all heritable factors as opposed to environmental controls on the expressed phenotype of an individual. Such information provides a critical first step toward understanding the biological potential for corals to adapt to ocean acidification, how coral communities may change in the future, and how to focus further research.

1.5 Study Significance

Hawaiian reefs provide a habitat for fish and invertebrates as well as over 500 species of algae that feed and provide oxygen for marine life. About one-fourth of the plants, fish, and invertebrates found in Hawaiian coral reefs are endemic to Hawaiʻi (NOAA, 2016). Worldwide, 15 percent of all corals are predicted to go extinct within 20 years. However, the discovery of species that may adapt to the acidifying ocean could change the outlook for the marine food web in the Hawaiian Islands.

Phenotype is the result of both heritable and environmental influences, but only heritable variation can be passed on to offspring and thus governs the potential for a population to adapt under selective pressure. This thesis aims to quantify the variation in coral calcification tolerances to low pH (~7.6) and the heritability of any such variation among the eight dominant coral species of O‘ahu. These species are *Montipora capitata, Montipora patula, Montipora flabellata, Pocillopora damicornis, Pocillopora meandrina, Porites compressa, Porites evermanni,* and *Porites lobata*, which constitute >97% of the coral cover on Hawaiian reefs. The estimation of heritable variation for these species is an important step in the discovery of how Hawaiian reef ecosystems may change over time.

2.0 Methods

2.1 Sampling Locations

Between eight and fifteen colonies of each species (Figure 3) were collected from around O‘ahu (Figure 4). Corals were collected from a total of six different locations, spanning natural gradients in seawater chemistry, temperature, wave exposure, etc. (Table 1), to consider natural habitat variation within these populations. Sampling was restricted to sites where each species is relatively abundant (Table 2). Once collected, coral samples were allowed to acclimate in a common garden for approximately six months, thereby excluding short-term recent history as a factor in their responses.

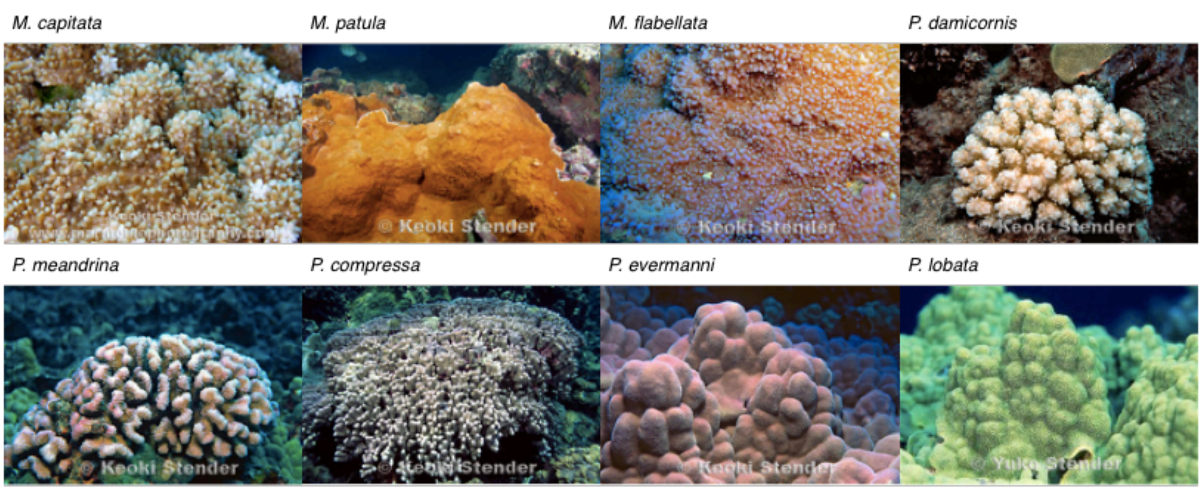


Figure 3. Images of the eight Hawaiian coral species studied. Source: Keoki Stender

Figure 4. Collection locations indicated by yellow stars.

Table 1. pH, temperature, and wave conditions at each collection location

Collection Location	pH	Temperature	Wave Exposure
Coconut Island	Low	High	Low
Haleʻiwa	High	Low	High
Kahe	High	Medium	High
Magic Island	High	Medium	Medium
Sampan Channel	Low	Medium	Medium
Waimānalo	High	Low	Medium

Table 2. Specific collection locations for each species.

	M. capitata	*M. patula*	*M. flabellata*	*P. damicornis*	*P. meandrina*	*P. compressa*	*P. evermanni*	*P. lobata*
Coconut Island	x			x		x		
Hale‘iwa	x	x	x	x	x	x	x	x
Kahe	x	x			x		x	x
Magic Island						x	x	x
Sampan Channel	x	x	x	x	x	x	x	x
Waimānalo	x	x			x	x	x	x

2.2 Coral Collection and Preparation

Corals were collected under permit SAP 2015-48, issued by the Department of Aquatic Resources (DAR) from a depth of 0.5-5 m with a hammer and chisel. 3 to 5 cm samples, called nubbins, were cut from each parent coral using a band saw to be distributed between treatments. Each nubbin was mounted on a labeled, plaster plug using cyanoacrylate gel (Figure 5b). Six replicate nubbins per colony were divided in four flow-through mesocosm (outdoor experimental system) tanks 300L, 160 nubbins per tank, arranged by species and grouped with nubbins from the same colony. Tank location did not have a significant effect on calcification rate. Tanks 1 and 3 were randomly selected as the low pH tanks, thus 2 and 4 were high pH tanks, discussed further in section 2.4.

2.3 Tank Monitoring and Chemistry

Each tank was covered by a shade cloth (Figure 5a) to give the corals about 60% shade, with an irradiance of about 800μmol photon m^{-2} s^{-1}, similar to conditions in their natural habitat. Tanks had constant seawater flow-through with one pump per tank for circulation. Tanks 1 and 3 were dosed with CO_2 gas to achieve pH levels of approximately 7.6, while high pH tanks received ambient seawater (pH ~8.0). Biweekly measurements of water pH, alkalinity, temperature, and salinity were taken from each tank at 12pm. Temperature and salinity values were measured with a YSI conductivity meter, alkalinity was measured using an autotitrator, and pH was assessed spectrophotometrically with m-cresol purple, following standard protocols (DOE, 1994). Additional carbonate chemistry parameters were calculated with CO2SYSTEMS (Lewis and Wallace, 1998). Tanks were cleaned twice per week to remove excess cyanobacteria and algae, which would normally be eaten or removed by reef creatures, to prevent excess shade or suffocation.

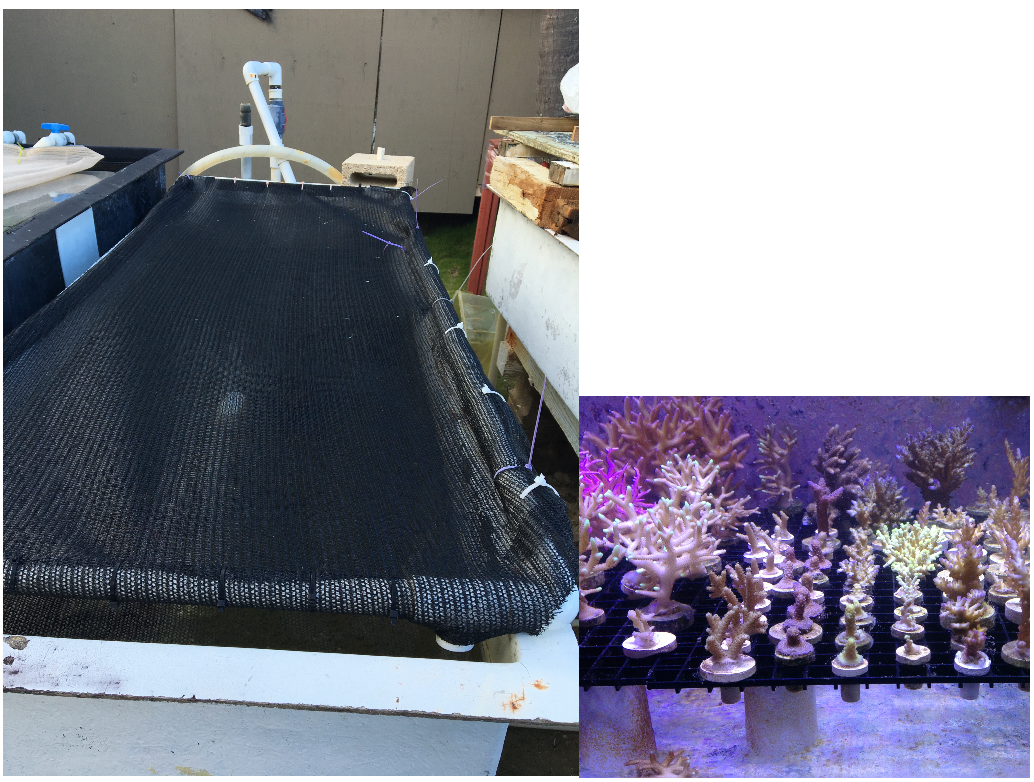


Figure 5. a) Image of one 300L tank with shade cloth cover. (left) b) Example image of corals on plaster plugs. (right)

2.4 Statistical Analyses

Coral calcification responses to the treatments were assessed using "repeatability" function in an R package called "heritability" (Kruijer et al. 2015). This ANOVA (Table 4) was used to estimate the genotypic and environmental variance components of broad sense heritability ($H^2=V_g/(V_g+V_e)$) based on the mean squared error (MSE) from ANOVA. 'Line repeatability' was set to false in order for the program to estimate H^2 from individual nubbins, as opposed to pooling within each genotype. The package estimates 95% confidence intervals for heritability. For these analyses the nubbins from each colony were pooled to estimate H^2 with pH set as an additional covariate. Hence, the heritability of calcification rate for each species was estimated taking into account the effect of pH on their calcification rates. The results are a single H^2 estimate for each species, along with 95% CI. Heritability values range from 0.0 to 1.0, with values indicating what proportion of the phenotypic variation that can be attributed to heritable factors.

The second ANOVA (Table 5) was run to assess the effect of pH and of colony on calcification rates of each species. The effect of pH x colony was also assessed, and significant pH x colony interactions indicate that the absolute reduction in calcification under low pH is statistically different from one colony to another, and some coral colonies were significantly more sensitive to acidification than others. pH and parent colony were fixed factors with tank as a nested factor. All analyses were performed with R v.3.1.2 (R Core Team, 2014); corals that experienced ≥50% tissue death were dropped from the analyses.

3.0 Results

3.1 Environmental Conditions

Table 3. Mean values and standard deviations for each parameter by tank.

Tank	pH	Temperature (°Celcius)	Alkalinity (μmol/kg)	Salinity (psu)	pCO_2 (μatm)	Ω Aragonite
1	7.619±0.068	25.6±0.6	2142±28	34.4±0.4	1168±199	1.41±0.21
2	8.026±0.033	25.6±0.58	2142±27	34.4±0.4	391±36	3.08±0.22
3	7.646±0.063	25.5±0.60	2145±27	34.4±0.4	1093±186	1.49±0.20
4	8.041±0.034	25.6±0.61	2141±30	34.4±0.4	375±38	3.17±0.21

Carbonate chemistry was successfully controlled during the experiment maintaining the average low pH levels at 7.619±0.068 and 7.646±0.063 and the ambient pH levels at 8.026±0.033 and 8.041±0.034 (Table 3). Omega (Ω) Aragonite is a measure of the saturation state of aragonite, the mineral from which corals skeletons are built.

3.2 HERITABILITY ESTIMATES

Table 4. H^2 values of each species. Columns correspond to ANOVA results.

Species	H^2	95% CI for H^2	Genotypic Variance	Environmental Variance	Effective # Replicates
M. capitata	0.562	0.350-0.782	1.567	1.219	5.932
M. patula	0.364	0.160-0.637	0.445	0.776	5.863
M. flabellata	0.484	0.201-0.821	3.331	3.550	6
P. damicornis	0.538	0.246-0.849	0.836	0.716	5.612
P. meandrina	0.682	0.488-0.854	1.121	0.521	5.730
P. compressa	0.580	0.371-0.793	1.576	1.139	6
P. evermanni	0.314	0.110-0.591	0.443	0.966	5.104
P. lobata	0.440	0.229-0.697	1.038	1.319	5.932

Analyses were repeated without pH as a covariate to assess if pH is a significant contribution to environmental variance, and although the heritability value was slightly lowered, the results were not statistically significantly different.

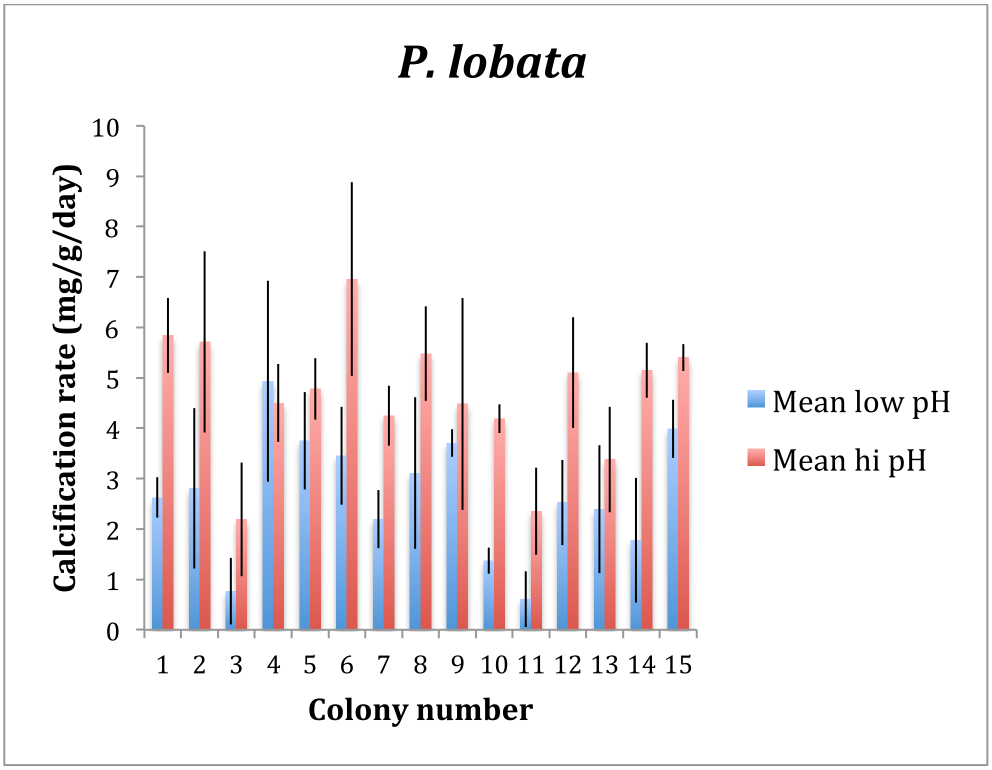
3.3 Anova Results

Table 5. ANOVA results for treatment effects of pH and coral colony on calcification rates for each species. DF = degrees of freedom; Sum sq = sum of squares; Mean Sq= mean of squares; F= variation between sample means / variation within the samples. P-values in bold are significant at the alpha = 0.05 level.

	DF Sum	Sum Sq	Mean Sq	F value	Pr(>F)
M. capitata					
pH	1	20.93	20.927	16.869	**p<0.001**
Colony	14	148.03	10.574	8.523	**p<0.001**
pH:Colony	14	15.80	1.129	0.910	p=0.55
Residuals	59	73.19	1.241		
M. patula					
pH	1	32.34	32.34	54.464	**p<0.001**
Colony	14	47.40	3.39	5.701	**p<0.001**
pH:Colony	14	21.44	1.53	2.578	**p<0.05**
Residuals	58	4.44	0.59		
P. meandrina					
pH	1	15.46	15.455	38.544	**p<0.001**
Colony	14	100.04	7.146	17.820	**p<0.001**
pH:Colony	14	14.05	1.004	2.503	**p<0.05**
Residuals	56	22.46	0.401		
P. evermanni					
pH	1	13.26	13.261	16.466	**p<0.001**
Colony	15	48.16	3.211	3.982	**p<0.001**
pH:Colony	14	21.73	1.552	1.925	**p<0.05**
Residuals	51	41.12	0.806		
M. flabellata					
pH	1	33.81	33.81	9.664	**p<0.01**
Colony	7	164.77	23.54	6.727	**p<0.001**
pH:Colony	7	26.52	3.79	1.083	p=0.39
Residuals	32	111.97	3.50		
P. damicornis					
pH	1	20.65	20.651	26.421	**p<0.001**
Colony	7	33.35	4.765	6.096	**p<0.001**
pH:Colony	7	3.14	0.449	0.574	p=0.77
Residuals	29	22.67	0.782		
P. compressa					
pH	1	18.94	18.942	16.865	**p<0.001**
Colony	14	148.36	10.597	9.435	**p<0.001**
pH:Colony	14	11.75	0.839	0.747	p=0.71
Residuals	60	67.39	1.123		
P. lobata					
pH	1	90.32	90.32	76.311	**p<0.001**
Colony	14	102.52	7.32	6.187	**p<0.001**
pH:Colony	14	26.46	1.89	1.597	p=0.10
Residuals	59	69.83	1.18		

The results of the ANOVA indicate that calcification by each of the eight species was significantly ($p<0.01$) affected by pH and by colony. The species *M. patula*, *P. meandrina*, and *P. evermanni* also show significant ($p<0.05$) pH x Colony interactions. For corals with no significant interaction among these factors, it means that the factors of pH and colony are additive, or not significantly different from additive. For *M. patula*, *P. meandrina*, and *P. evermanni,* the significant pH x Colony interactions indicate that the absolute reduction in calcification under low pH is statistically different from one colony to another, and some coral colonies were significantly more sensitive to acidification than others.

Figure 6 shows two graphs, each depicting average growth rate per colony in low pH and high pH. The first graph, figure 6a, is for *Porites lobata*, but is also a good depiction of the general affect of low pH on calcification rate. The second graph, figure 6b, is of *Montipora patula*, one of the three species that showed varying sensitivities to low pH.



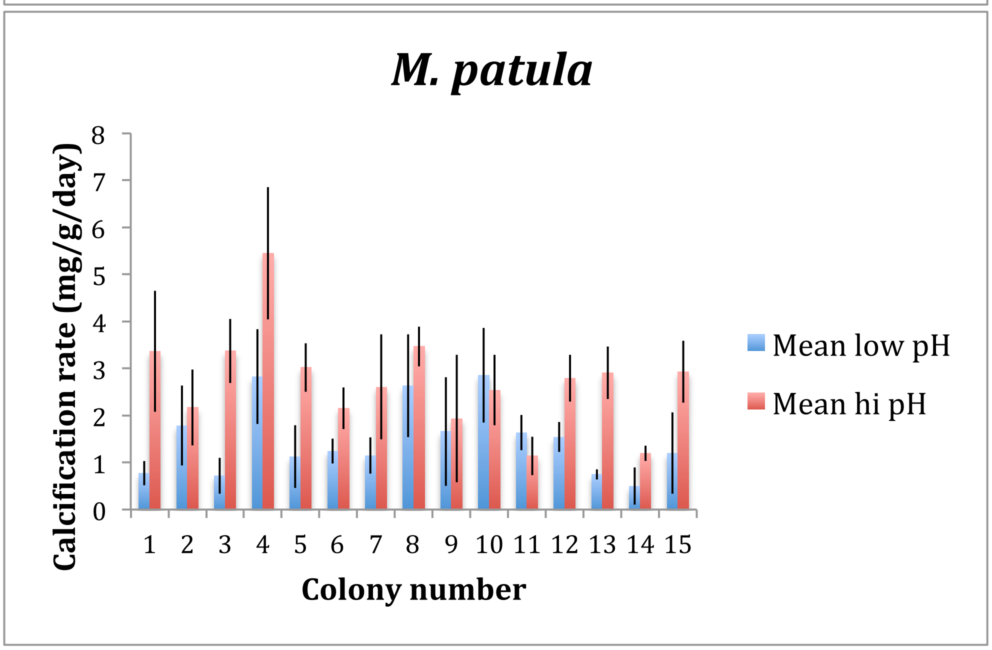


Figure 6. a) Mean calcification rate for each *P. lobata* colony (top). b) Mean calcification rate for each *M. patula* colony (bottom).

4.0 Discussion

4.1 Candidates For Adaptation

Calcification rates are significantly heritable across all eight species, and all eight may experience selective pressure for calcification rate under OA. *M. patula*, *P. meandrina*, and *P. evermanni* in particular show statistically significant variation in pH tolerance among colonies, making these especially good candidates for future studies of OA adaptation. However, there could be tradeoffs to different calcification rates, discussed in section 4.3.

All eight species were found to have unusually high heritability rates, as heritability values of life-history traits, traits that are connected to a species' fitness, tend to have lower heritability than morphological traits (Price & Schluter, 1991; Moussea & Roff, 1987). Life-history heritability values are often low because populations are expected to be near evolutionary equilibrium with low genetic variance in total fitness. The high heritability values for these species suggest that a large portion of the observed variation in calcification rates among colonies is due to biological factors. This is a hopeful discovery for Hawaiian reefs, as the pressure of OA could act upon the genetic variation within these populations and drive adaptation, thus producing more tolerant corals.

4.2 Coral Acclimatization

It is important to address the notion of acclimatization as it relates to the experiment. The possibility that acclimatization over the long term is responsible for the variation in calcification rates has been studied, but the results shown no evidence that they can do so. For example, a study conducted in 2008 tested coral growth rates every two weeks under acidified conditions but concluded that over several months, measurements failed to produce a trend that suggested an acclimation response to OA (Jokiel et al, 2008). Similarly, an experiment on corals under acidified conditions also tested for possible long-term acclimation by measuring internal pH responses made by the corals at the site of calcification after 24 hours under treatment conditions and after one year of exposure. The study concluded that the responses over both timeframes were very similar, and coral colonies were not adjusting to OA over the course of the year (Venn et al, 2013). Though these previous studies only tested responses of two species (including one of the eight species examined here), there is little evidence suggesting that corals can acclimate over time to acidification.

It is possible that the ability to acclimatize is limited to early in the life cycle and development within a given environment leads to channelization of the physiological responses of the organism for the rest of their life. It is also possible that the coral species in this study had greater capacity to acclimatize than those examined previously. The possibility that the variation among coral colonies measured could be driven by long-term acclimatization and not local adaptation is considered in the data interpretation. Even if some or all of this variation is driven by acclimatization, however, the results still allow the estimation of broad sense heritability and will provide critical information that can be used to guide future studies examining the mechanistic basis of this variation.

4.3 Overall Growth Variation

For many species, there were large differences in calcification rates from colony to colony (see Appendix B). There are many physiological and ecological trade offs that should be considered when interpreting these variations. Growth rate determines competitiveness, as a faster growth rate means more coral cover per unit area and a better chance for reproduction. However, a fast growth rate may detract energy that might otherwise be used by the coral to fight disease or other stressors.

A 2014 study, conducted to find properties of corals that are sensitive and resistant to OA, found that fast calcifiers were more sensitive to low pH than slower ones (S. Comeau et al., 2014). Fast calcifiers (defined operationally as those that calcified at rates of > 1 mg/cm2/d) were more sensitive to acidification, in absolute terms, than slow calcifiers (< 1 mg/cm2/d). This study also found that effects of low pH were additive with coral species among colonies for several of the coral species (similar to the results in this thesis, Table 4). This indicates that the species that grow quickly experienced a large absolute reduction in calcification, and the species that grow more slowly experienced a lower absolute reduction in calcification. Although the study was comparing different species and this study compares different colonies within the same species, comparable results are seen.

4.4 Effect of OA on Calcifiers

It has been generally accepted that pCO_2 in the ocean lowers the aragonite saturation state, thus preventing corals from incorporating the mineral into their skeletons. Geochemically, it is obvious to conclude that the reason corals struggle to grow under OA is because of this. However, from a biological and physiological standpoint, it is less obvious that lack of free aragonite or calcite is preventing the corals or other calcifying creatures from calcifying. Physiologically, an aragonite pump or a comparable mechanism to take up free aragonite from the ocean and incorporate it into their skeleton has not been discovered within corals. Instead, studies have shown that, at the site of calcification, corals actually control pH by releasing protons (H^+) (Venn et al, 2013). The combination of dissolved inorganic carbon (DIC) and bicarbonate concentrations paired with the low pH might be a better explanation of reduced calcification. This assumption is hard to distinguish because aragonite saturation state and increased bicarbonate concentrations and DIC are correlated. If calcification is a predominantly biological mechanism that is impacted by environmental influences, then there is a potential for corals to adapt.

4.5 Relevant Studies

A yearlong study conducted by researchers at San Francisco State University found that calcifying coccolithophores *Emiliania huxleyi* were able to build their plated carbonate skeletons despite elevated pCO_2 and temperature conditions (average 833 ± 68 μatm and 24.0 ± 0.2°C) (Benner et al., 2013). 700 generations of coccolithophores were raised over the course of the experiment, and displayed adaptation to the warm, acidic water. A similar study (Lohbeck et al., 2012) found that after 500 generations in elevated pCO_2 conditions *E. huxleyi* cells that were adapted to higher pCO_2 showed up to 50% higher calcification rates than those adapted to ambient pCO_2. Benner et al. concluded that changes in calcification might not be explained by calcification-related genes as much as by intracellular regulatory processes. These studies show that the mechanisms used by calcifying organisms are more complex than is currently understood, and that higher pCO_2 may not directly influence organisms' calcification. This study also noted that there is much complexity regarding the future of the ocean in terms of light availability and nutrient concentration, both of which are critical factors in growth rate.

A 1990 a study conducted on Hawaiian corals' response to elevated temperature found that the upper lethal temperature for several Hawaiian corals including *Pocillopora meandrina*, *Pocillopora damicornis,* and *Porites lobata* is 32°C (Jokiel et al., 1990). At 31.3°C corals lost symbiotic zooxanthellae, suffered tissue damage, or died. The same species from a warmer environment, Enewetak, were able to withstand temperatures up to 35°C. The average Hawaiian sea surface temperature is around 25°C and could increase between 2-4 Celsius by 2100 (IPCC, 2014). It is critical to note that while some Hawaiian coral species may be able to adapt to the continual acidification of the ocean, sea surface temperature plays a large role in coral mortality.

5.0 Conclusion

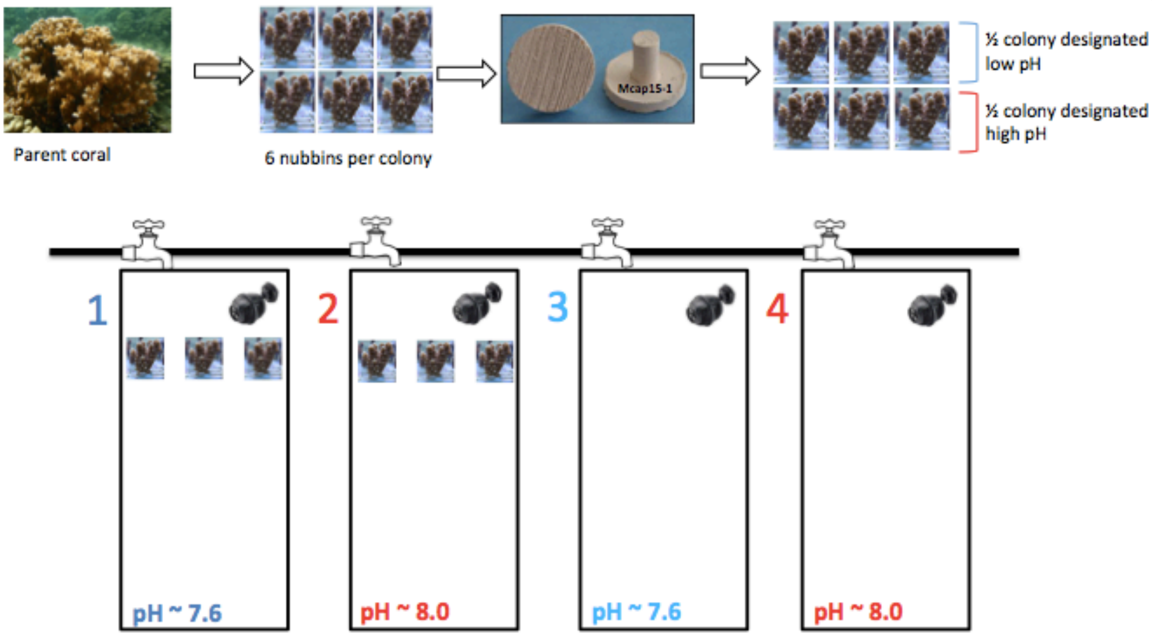
The heritability in pH tolerances identified is a critical discovery in Hawaiian coral reef conservation. As coral reefs serve as the basis of vast ecosystems on which marine food webs depend, the identification of species that may be able to adapt as the ocean acidifies is a significant first step in planning for the future. These results offer insight into how coral communities may shift under ocean acidification and will help to guide future studies examining the mechanistic basis of coral responses to ocean acidification. This information can be passed on to the National Oceanic and Atmospheric Association (NOAA) or other protective agencies and included in their adaptive management plans.

Montipora patula, *Pocillopora meandrina*, and *Porites evermanni* are important species that may be particularly capable of adapting to thrive in waters of lower pH. These three species are phylogenetically diverse, representing three different coral families, Acroporidae, Pocilloporidae, and Poritidae, and both major evolutionary clades of corals, Complexa and Robusta. The complex and robust clades of corals split ~420 million years ago, and these coral families split >100 million years ago, meaning that even among our limited taxonomic sampling, species from several distinct lineages are showing strong potential to adapt to OA. This capacity to adapt is potentially widespread and many other unexamined corals might have similar potential.

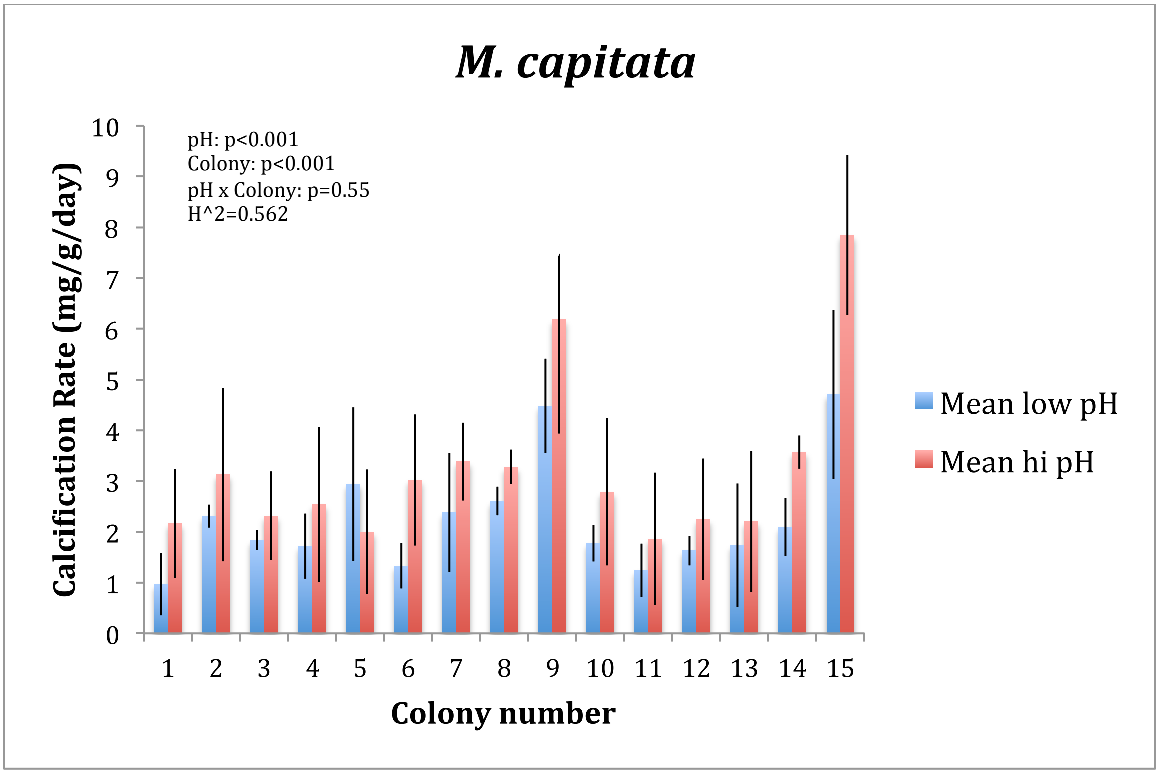
Ocean acidification is not the only factor in coral calcification, but also temperature, light availability, geographic location, and nutrient concentrations. A follow up study should be conducted to assess the success of these eight coral species under the predicted future combination of the aforementioned factors as well as OA, in order to achieve a more comprehensive understanding of what might happen to these reefs in the future.

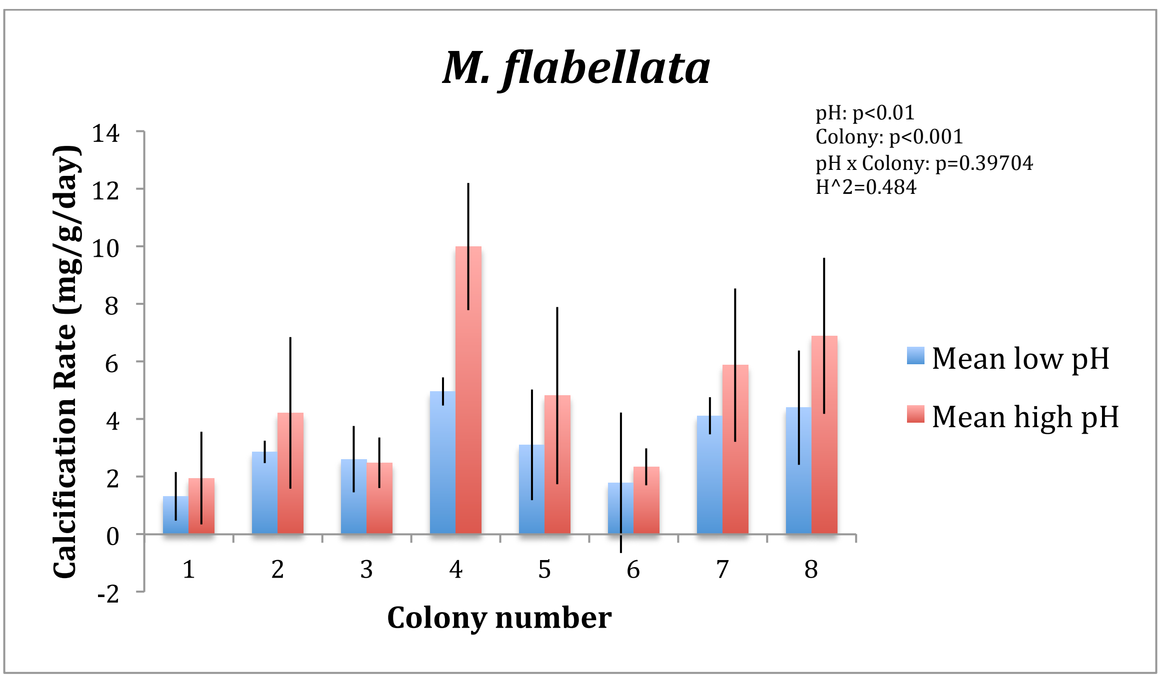
This study indicates that overall OA has a negative effect on Hawaiian coral growth rates though the mechanism is still unclear; yet the high heritability values found in this thesis provide a hopeful outlook for the reef composition in the future.

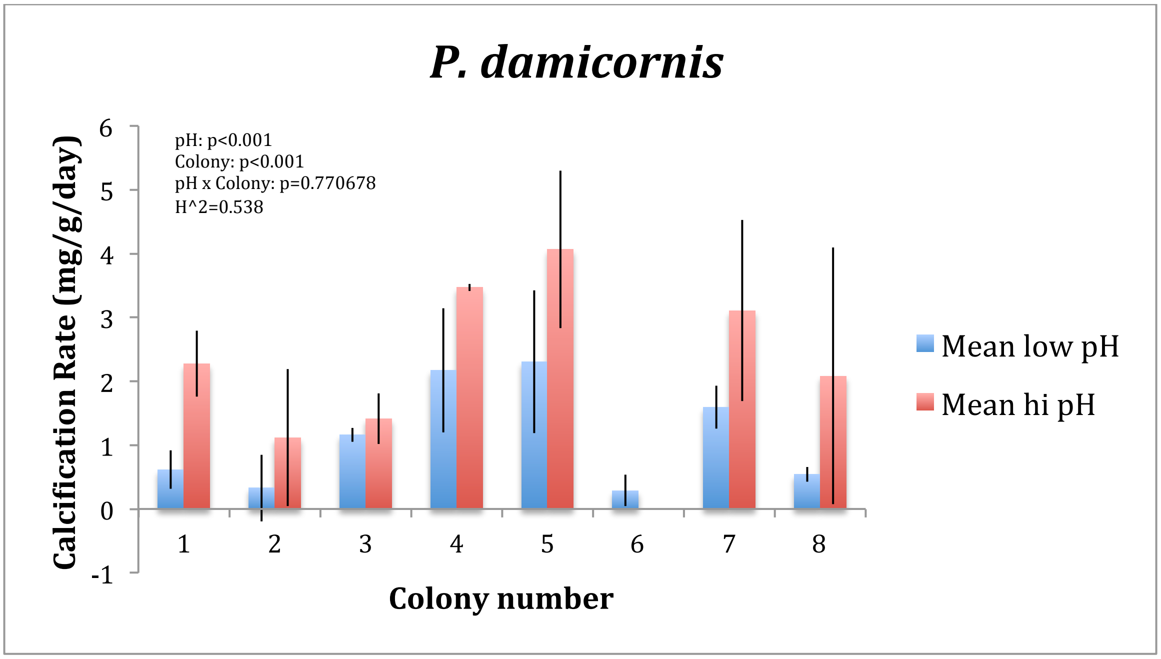
Appendix A: Experimental design graphic

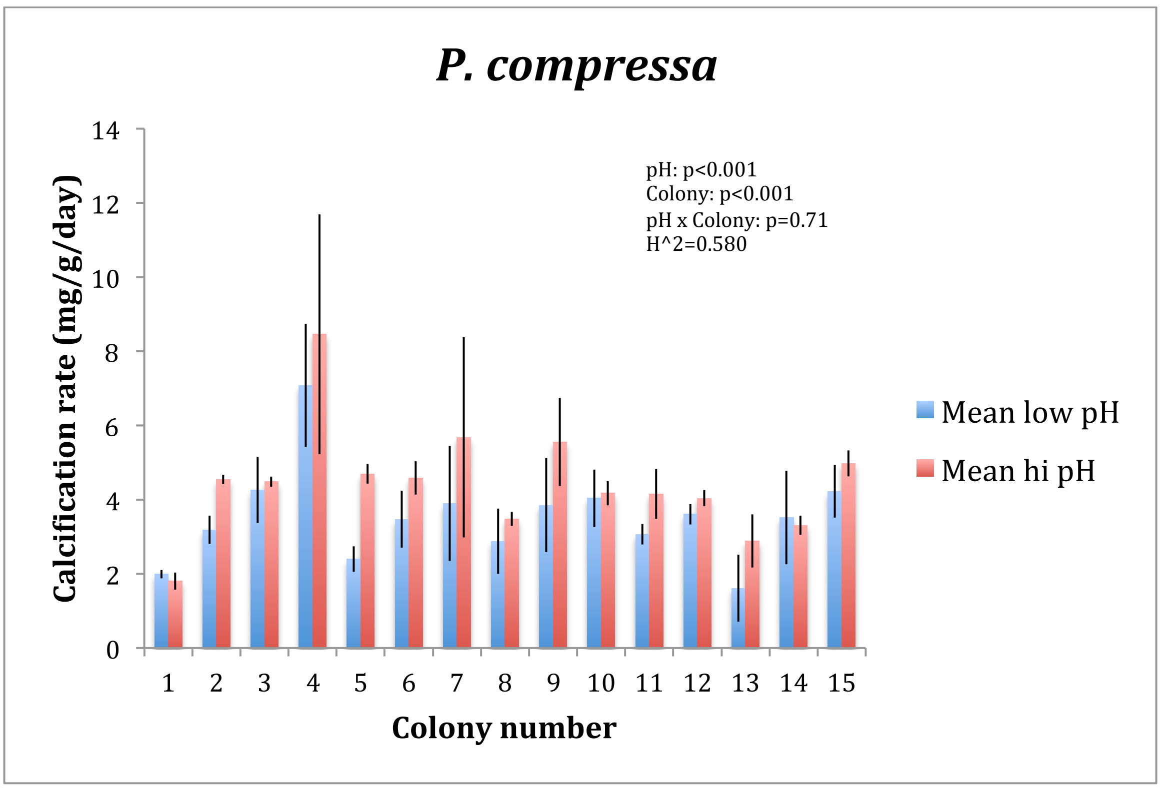


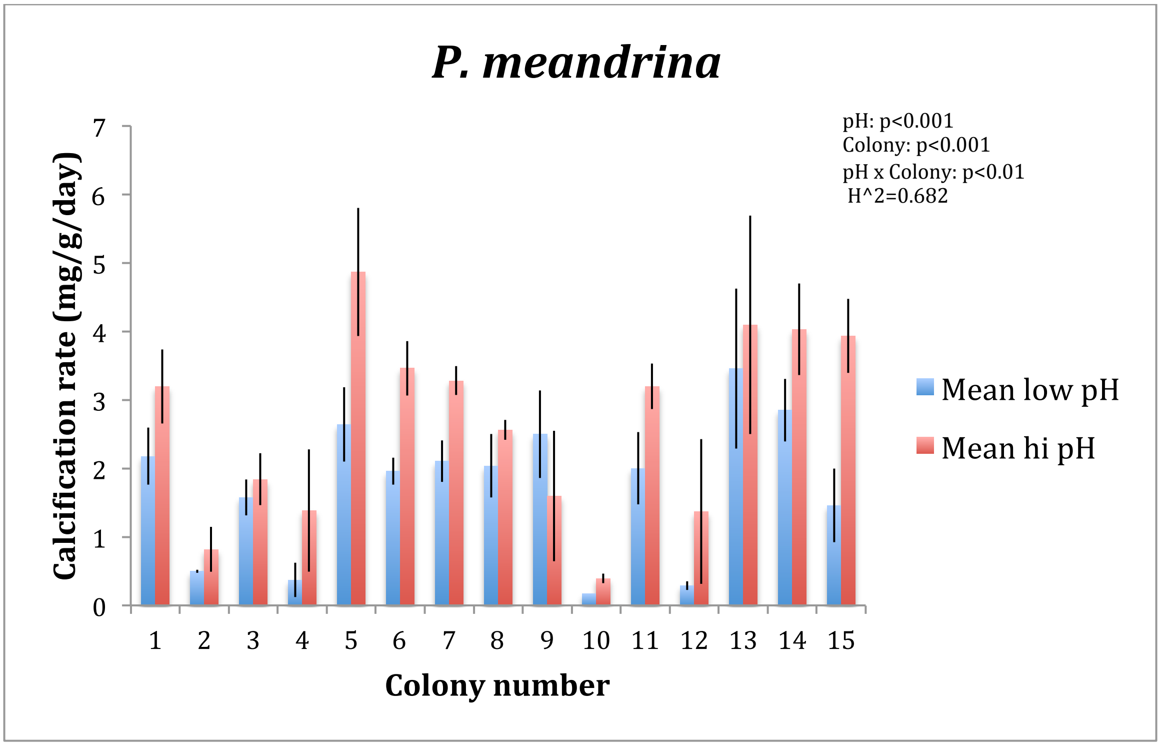
Appendix B: Graphs of mean calcification rates with standard deviation bars for each species.

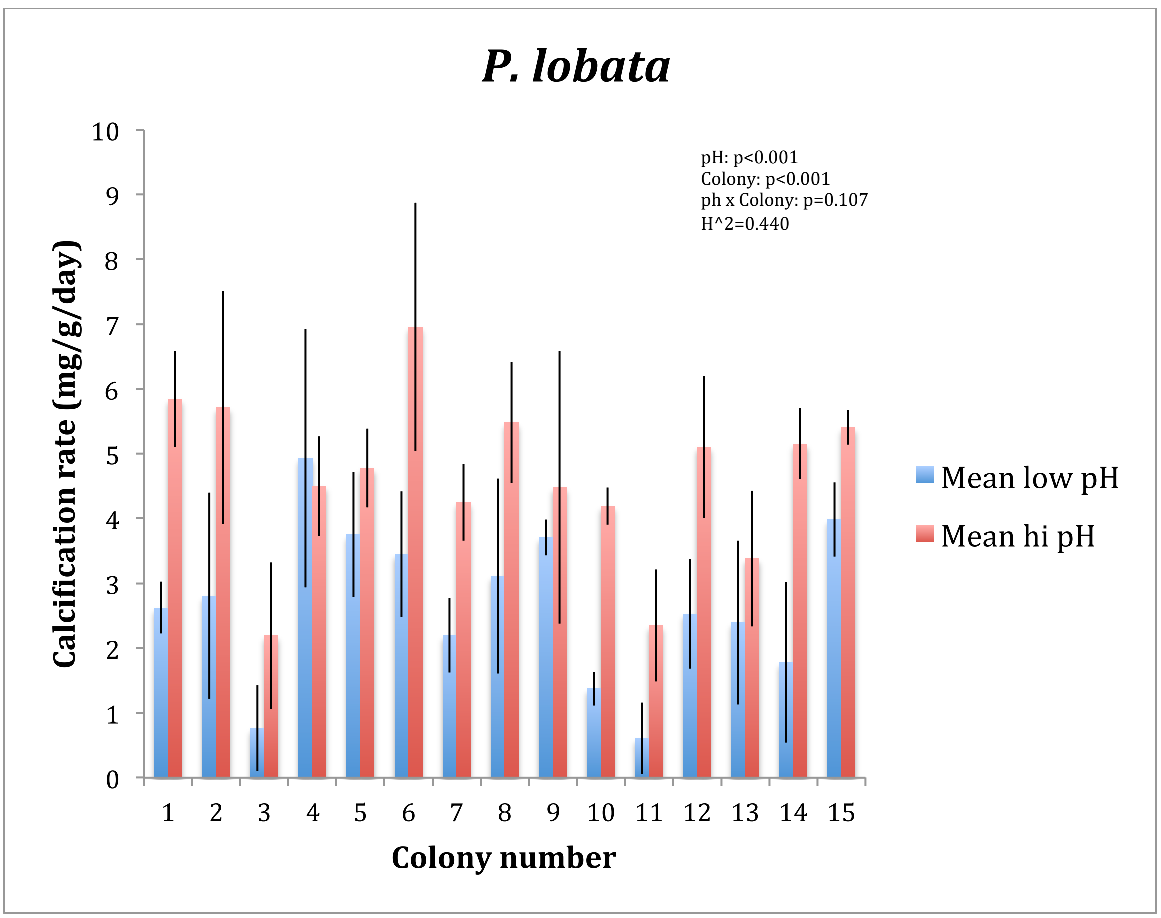


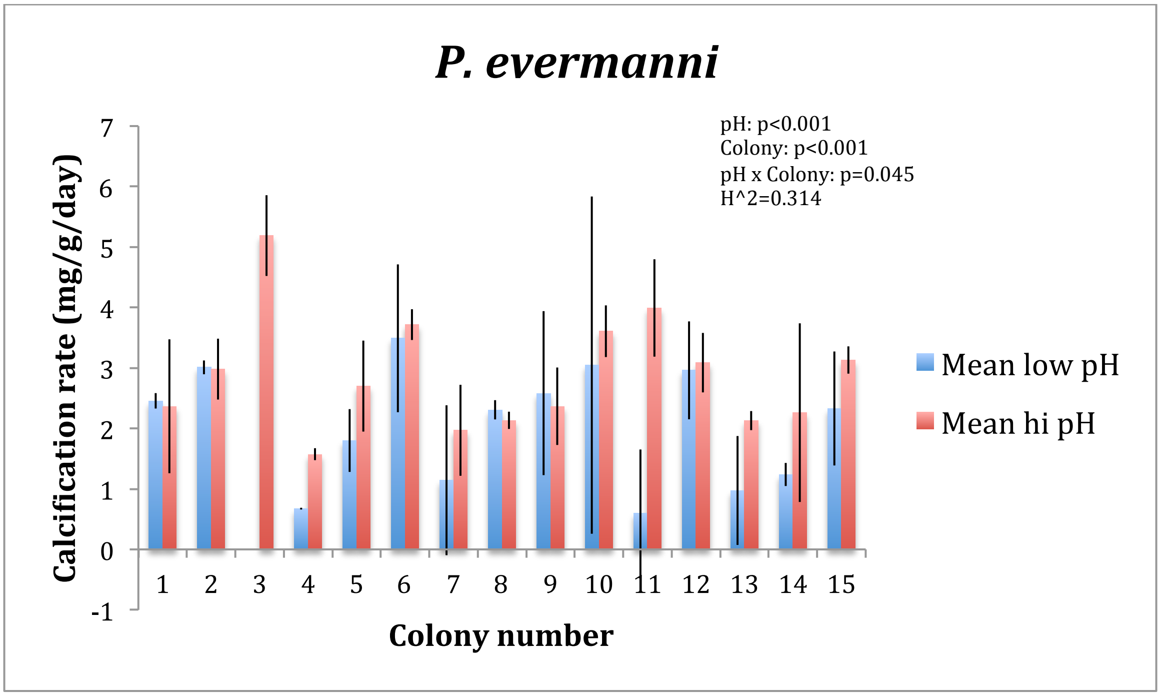


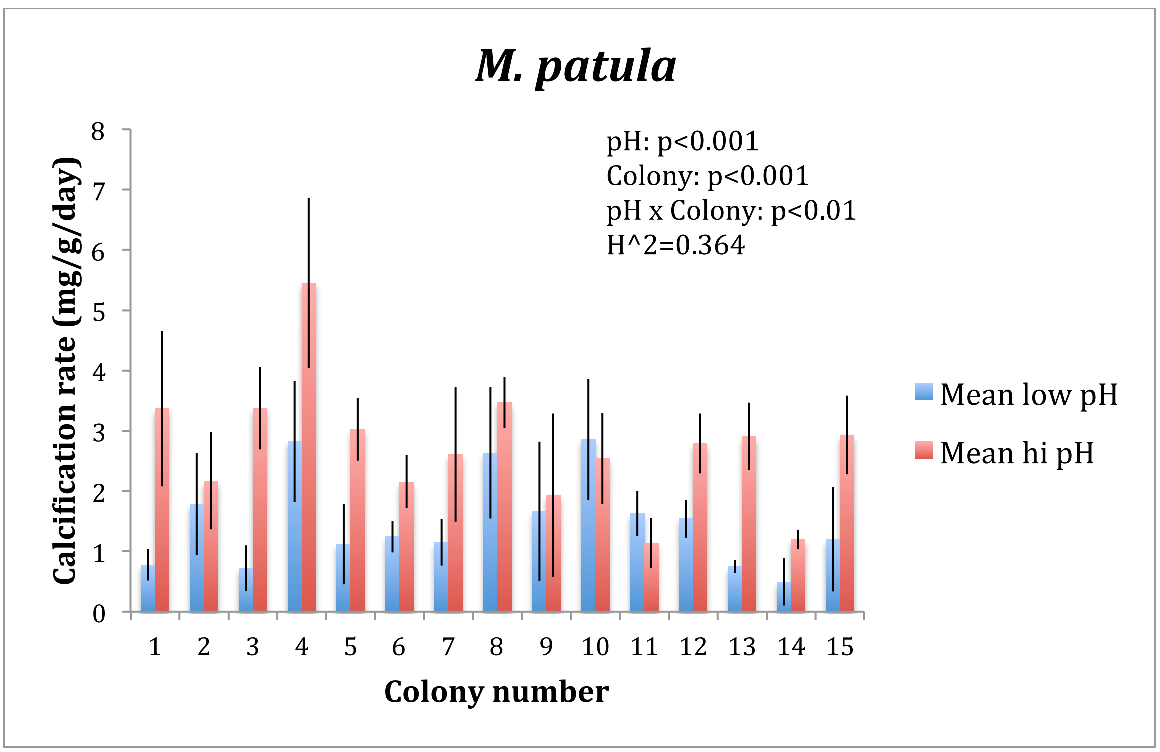

P. damicornis
pH: p<0.001
Colony: p<0.001
pH x Colony: p=0.770678
H^2=0.538
Calcification Rate (mg/g/day)
6
5
4
3
2
1
0
-1
1
2
3
4
5
6
7
8
Colony number
Mean low pH
Mean hi pH


P. compressa
pH: p<0.001
Colony: p<0.001
pH x Colony: p=0.71
H^2=0.580
Calcification rate (mg/g/day)
14
12
10
8
6
4
2
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Colony number
Mean low pH
Mean hi pH


P. meandrina
pH: p<0.001
Colony: p<0.001
pH x Colony: p<0.01
H^2=0.682
Calcification rate (mg/g/day)
0
1
2
3
4
5
6
7
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Colony number
Mean low pH
Mean hi pH


P. lobata
pH: p<0.001
Colony: p<0.001
ph x Colony: p=0.107
H^2=0.440
Calcification rate (mg/g/day)
0
1
2
3
4
5
6
7
8
9
10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Colony number
Mean low pH
Mean hi pH


P. evermanni
pH: p<0.001
Colony: p<0.001
pH x Colony: p=0.045
H^2=0.314
Calcification rate (mg/g/day)
7
6
5
4
3
2
1
0
-1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Colony number
Mean low pH
Mean hi pH


M. patula
pH: p<0.001
Colony: p<0.001
pH x Colony: p<0.01
H^2=0.364
Calcification rate (mg/g/day)
8
7
6
5
4
3
2
1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Colony number
Mean low pH
Mean hi pH

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